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(54) Title: METHODS AND COMPOSITIONS FOR HAIR GROWTH PROMOTION

(57) Abstract

Methods and composition for promoting hair growth rely on restoring or enhancing the activity of lymphoid-enhancer factor-1 in hair follicles. The activity may be restored by applying a composition including LEF-1 present in a vehicle in an amount sufficient to restore or enhance hair growth promotion when applied to a region of a patient's skin. Alternatively, the methods may comprise applying a composition comprising all or a portion of the LEF-1 gene present in a suitable vector, such as a liposomal or retroviral vector.

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# METHODS AND COMPOSITIONS FOR HAIR GROWTH PROMOTION

## BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to methods and compositions for promoting hair growth and, more particularly, to compositions and methods which restore or enhance the activity of LEF-1 and proteins regulated by LEF-1 in hair follicles.

Alopecia, more commonly referred to as baldness, can result from a variety of causes, including genetic factors, aging, local and systemic diseases, and trauma. The most common form of alopecia is referred to as "male-pattern baldness," an apparently genetic condition which is characterized by increasing hair loss with advancing age. At present, there are no known cures for any form of alopecia. The most effective therapy is transplantation of hair follicles from hairy to bald areas of the skin. The use of minoxidil, anti-hypertensive drug, has been approved for the treatment of male-pattern baldness, but significant hair growth as the result of topical application of minoxidil is very rare.

It would therefore be desirable to provide improved methods and compositions for promoting hair growth in patients suffering from alopecia. It would be particularly desirable to provide compositions incorporating active agent(s) which are effective when topically applied to the skin in a region where hair growth is desired. It would be even more desirable to provide such methods and compositions which are effective in treating male-pattern baldness as well as other common forms of alopecia.

2. Description of the Background Art

Methods and compositions for promoting hair growth are described in numerous patents and publications. See, for example, U.S. Patent No. 5,407,944; WO 95/08328; EP 656 201; and DE 43 23 616. The latter discloses compositions comprising nucleic acids. WO 94/22468 describes liposomal vehicles for delivery genes and other substances to hair follicles.

Lymphoid enhancer factor 1 (LEF-1) and its biological activities are described in a number of publications, including Giese et al. (1995) GENES & DEV. 9:995-1008; Giese and Grosschedl (1993) EMBO J. 12:4667-4676; Giese et al. (1992) CELL 69:185-195; Giese et al. (1991) GENES & DEV. 5:2567-2578; and Travis et al. (1991) GENES & DEV. 5:880-894.

LEF-1 expression has been associated with the growth of hair. Van Genderen et al. (1994) GENES & DEV. 8:2691-2703 and Zhou et al. (1995) GENES & DEV. 9:570-583. Data presented in van Genderen et al. (1994) form part of the present application. Van Genderen et al. (1994) was published less than one year prior to the filing date of the present application.

## SUMMARY OF THE INVENTION

The present invention is based at least in part on the discovery that expression of lymphoid enhancer factor-1 (LEF-1) is essential for the development of hair follicles and the growth of hair from hair follicles. The method of the present invention thus comprises restoring or enhancing the activity of LEF-1 in hair follicles in the epidermis (skin) of patients suffering from alopecia.

According to a first aspect of the method of the present invention, a composition comprising LEF-1 and/or a biologically equivalent substance is provided. The composition is then applied to a region of skin, typically the scalp, where hair growth promotion is

desired. LEF-1 may be in the form of the full length LEF-1 protein, or an active portion thereof, typically retaining at least the HMG box of the protein, more typically being in recombinant form. Alternatively, the LEF-1 substance may be an analog, mimetic, or other molecule which displays a similar or identical activity to LEF-1 in promoting hair follicle generation and hair growth therefrom.

In a second aspect of the method of the present invention, LEF-1 activity is restored or enhanced by providing a composition comprising an LEF-1 gene in a topical carrier. The composition is then applied to the skin where hair growth promotion is desired. The LEF-1 gene will typically encode the full length LEF-1 gene product or an active portion thereof, and will usually be delivered as part of a liposomal or retroviral vector. The gene will usually be provided in reading frame with homologous or heterologous regulatory control sequences.

Compositions according to the present invention comprise an LEF-1 polypeptide or LEF-1 gene in a topical carrier. In the case of LEF-1 polypeptides, either the full length LEF-1 protein or a fragment, analog, or mimetic thereof may be employed. In the case of the LEF-1 gene, the gene will usually be present in a liposomal or retroviral vector, and the gene will generally be in reading frame with homologous or heterologous regulatory control sequences. The LEF-1 polypeptide and/or gene may be derived from or based on the reported sequences of murine, human or other mammalian sources. Specifically, it has been found that the murine form of LEF-1 is active in human cells.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the pattern of expression of LEF-1 during mouse development. In situ hybridization and immunohistochemistry analysis of sagittal sections of frozen mouse embryos at days 12.5, 14.5, and 16.5 of gestation. Embryo sections were hybridized with a $^{35}S$-labeled LEF-1 anti-sense probe, and sites of expression were visualized by dark-field illumination. The specificity of hybridization was confirmed by using a LEF-1 sense RNA probe (data not shown). (A) Sagittal section of an E12.5 embryo. Sites of abundant LEF-1 expression include mesencephalon (me), diencephalon (d), inner ear (ie), tongue (t), lower and upper lips (ll, ul), nasal process (np). (B) Mid-sagittal section of an E14.5 embryo. Sites of LEF-1 expression include pituitary gland (p), tooth buds (tb), and the kidney (k), (te) Telencephalon. (C, D) Higher magnification of a mandibular tooth germ at early bud stage (E12.5) and at cap stage (E14.5), respectively. At E12.5, LEF-1 expression is localized predominantly to the mesenchyme (ms) underlying the presumptive dental epithelium (e), but later (E14.5) it is localized to both the dental papilla mesenchyme (dp) and dental epithelium (e). The position of Meckel's cartilage (Mc) is indicated. (E) Protein expression of LEF-1 in a mandibular tooth germ at the cap stage (E14.5) was visualized by immunohistochemistry. Bar, 100$\mu$m. (F) LEF-1 RNA expression in the whisker hair follicles, termed vibrissae (v), of an E16.5 embryo. (G) LEF-1 RNA expression in the mesencephalon (me) of an E12.5 embryo. (H) Localization of LEF-1 protein in the mesencephalon of an E12.5 embryo by immunohistochemistry. LEF-1 is expressed in the nuclei of large cells that constitute the developing TMN. Bar, 50$\mu$m.

Fig. 2 depicts the scheme of a targeted disruption of the murine LEF-1 locus. (A) The LEF-1 cDNA with the HMG domain shown as a solid box is indicated at

the top. The line below represents the wild-type LEF-1 locus in the region of the HMG domain. The targeting vector includes a PGK-neo$^r$ gene, inserted into the *Sma*I site residing in the 3′ exon of the HMG domain, and a *tk* gene. Transcriptional polarity of the neo$^r$ and *tk* genes are indicated by arrows. pBR vector sequences are represented by a shaded box. Sites for restriction enzymes are indicated above the lines, and the length of the fragments generated by a *Bam*HI-*Eco*RI digest that hybridize with a genomic flanking probe are indicated below. (B) DNA blot analysis of genomic DNA from a representative litter generated by the mating of heterozygous mutant LEF-1-deficient mice. DNA was digested with *Bam*HI-*Eco*RI and probed with a fragment shown in A. The 12.9- and 4.0-kb DNA fragments are generated from the wild-type and mutant LEF-1 allele, respectively. (C) Immunoblot analysis of nuclear extract from pre-B cells that were obtained from wild-type, heterozygous, and homozygous mutant LEF-1 deficient mice by transformation with Abelson MuLV. The blot was incubated with purified polyclonal antibodies directed against LEF-1 as described in Travis et al. (1991) Genes & Dev. 5:880-894, and LEF-1 was visualized by an alkaline phosphatase secondary antibody. The position of the predominant 55 kD LEF-1 polypeptide is indicated. A minor 39kD LEF-1 polypeptide is visible, which may be a degradation product or a product of an alternatively spliced LEF-1 transcript. Equivalent amounts of nuclear extract were used in each lane as confirmed by staining a parallel blot with Ponceau S (data not shown).

Fig. 3 shows a phenotype of LEF-1-deficient mouse. The homozygous mutant mouse (left) at day 16 after birth lacks whiskers and body hair. Moreover, the snout has a pointed appearance and the animal is significantly smaller compared to a wild-type sibling mouse (right).

Fig. 4 shows skin from wild-type (+/+) and mutant (-/-) postnatal day 3 mice. (A, B) Paraffin sections through the skin in the head of a wild-type (A) and mutant (B) animal stained with hematoxylin-eosin. The skin from the mutant is considerably thinner, lacks dermal fat, and has fewer hair follicles. Bar, 200μm. (C, D) Plastic sections, stained with toluidine blue, showing the morphology of wild-type (C) and mutant (D) hair follicles. Hair follicles in the mutant are rudimentary and fail to project as deeply into the dermis as normal follicles. The hair sheath is not fully differentiated. The epidermis of the mutant, however, is histologically normal. It shows an apparently normal layering and keratinization. In contrast, the dermal adipocytes in the mutant are severely underrepresented. The brown pigment in wild-type hair follicles (A, C) corresponds to melanin granules not seen in mutant follicles (B, D). (c) Cortex; (dp) dermal papilla; (fc) fat cell; (hb) hair bulb; (hs) hair sheath; (is) inner sheath; (os) outer sheath. Bar, 50μm.

(E, F) Sections from wild-type (E) and mutant (F) mice immunostained with antibodies to c-kit, a marker for melanocytes. Note the normal presence of both epidermal (arrowheads) and dermal melanocytes. The lack of melanin in the mutant hair follicles is not attributable to a lack of melanocytes but to a failed melanogenic activity caused by the arrested hair growth cycle. (hf) Hair follicle. Bar, 100μm.

## DESCRIPTION OF THE SPECIFIC EMBODIMENTS

As used hereinafter and in the claims, the following terms and phrases are defined to have the meanings set forth.

The term "hair" and phrase "hair growth" refer to the emergence of keratinized cells from hair follicles. Hair follicles are tubular epidermal structures that penetrate into the dermis and have a

widened portion (the bulb) at their lower ends. Cell production within the matrix of the hair follicle results in the growth of a hair shaft which emerges from the skin surface.

The term "alopecia" is a clinical condition characterized by the partial or complete loss of hair from the patient. It may result from heredity, aging, local or systemic disease, or trauma. The most common form of alopecia to be treated by the present invention is male-pattern baldness which is generally a hereditary condition. Other forms of alopecia which may be treated include toxic and traumatic forms of alopecia. The present invention will be particularly useful for treating alopecia occurring on the scalp, but in some cases may also find use with other regions of the dermis.

The phrase "promoting hair growth," includes both restoring hair growth in patients where hair growth is entirely absent and enhancing the rate of hair growth in patients where hair is growing at a less than desired rate. Such hair growth promotion may be effected on any region of the dermis where hair follicles are present or capable of being replaced, but will most usually be performed on the scalp.

The phrase "lymphoid enhancer-binding factor 1" and designation "LEF-1" refer to a mammalian cell type-specific transcription factor that is expressed in lymphocytes and sites of organogenesis and responsible for a variety of activities. Specifically, based on the discovery described in the present application, LEF-1 is expressed in the epidermis and in hair follicles and is responsible for the embryogenic emergence of hair follicles. LEF-1 is a member of the family of high mobility group (HMG) proteins, which have the capacity to induce a sharp bend in the DNA helix and to activate transcription only in collaboration with other DNA-binding proteins. The cloning of the murine LEF-1 gene

and full gene sequence are provided in Travis et al. (1991), *supra*. The murine gene sequence together with corresponding amino acid sequence are also set forth in SEQ ID. No. 1 and SEQ ID. No. 2, respectively. Specific methods for expressing full length LEF-1 protein and various amino-terminal truncations thereof are described in Giese and Grosschedl (1993), *supra*. The methods and compositions of the present invention can utilize either the murine, human, or other mammalian LEF-1 gene and proteins. The sequence, cloning, and expression of human LEF-1 are described in Waterman et al. (1991) GENES & DEV. 5:656-669.

Compositions according to the present invention which incorporate the LEF-1 gene or a portion thereof may be prepared as follows. A DNA segment is prepared which encodes an amino acid sequence corresponding entirely or partially to at least an active fragment of the LEF-1 gene product as set forth in SEQ ID No. 2. Usually, the amino acid sequence will comprise at least the HMG box, and more usually comprise the entire wild-type protein. It will be appreciated, however, that variations of one or several amino acids, and in some cases deletions of relatively large inactive portions of the protein, may readily be made without significantly altering the biological activity of the protein expressed by the DNA sequence. Usually, the DNA segment will have a sequence which corresponds to the nucleotide sequence set forth in SEQ ID No. 1, but this is not necessary because of the redundant nature of the genetic code. That is, a variety of different codons which code for the same amino acids may be substituted for those found in the wild-type LEF-1 gene.

Once prepared, the DNA segment which encodes the LEF-1 protein or fragment thereof will usually be combined with homologous or heterologous regulatory control sequences which enable expression of the gene after transduction into the host cells of the patient.

The recombinant DNA segments incorporating both the LEF-1 coding sequence and the homologous or heterologous regulatory control sequences will then be incorporated into a suitable delivery vehicle for transduction of the host cells by topical application. Useful transduction vehicles include both liposomal vehicles and retroviral vectors, both of which are well described in the patent and scientific literature. Suitable liposomal vehicles are described in, for example, U.S. Patent Nos. 5,223,263; 5,264,618; 5,077,211; 5,064,655; 5,043,164; 4,957,735; 4,954,345; 4,925,661; 4,828,837; 4,755,388; 4,529,561; 4,394,448; and 4,241,046; and PCT publications WO 95/12387 and WO 94/22468. Suitable retroviral vectors are described in PCT publications WO 95/14102; WO 95/14101; WO 95/07994; WO 95/06743; WO 94/28938; and WO 94/23751. The full disclosures of each of these references describing liposomal and retroviral delivery vehicles are incorporated herein by reference. A preferred liposomal vehicle for delivering LEF-1 genes to hair follicle cells according to the method of the present invention is described in WO 94/22468.

Compositions incorporating LEF-1 polypeptides according to the present invention may be prepared as follows. The LEF-1 polypeptides may comprise full length LEF-1 proteins or active fragments, analogs, or mimetics thereof. Usually, the LEF-1 polypeptides will be recombinantly produced based on the amino acid sequence set forth in SEQ ID No. 2, using recombinant protein production techniques that are now widely described in the technical and scientific literature. See, for example, MOLECULAR CLONING: A LABORATORY MANUAL, Sambrook et al., Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989), vols. 1-3. Such recombinantly produced LEF-1 polypeptides will be purified to a high degree of purity using conventional protein separation techniques. Typically, a purity of at least about 90% by weight will be obtained, preferably at

least about 95% by weight, and more preferably at least about 99% by weight.

Both the LEF-1-gene-containing and LEF-1-protein-containing compositions will usually be incorporated in a carrier suitable for topical application to the skin, more typically the scalp. Such topical carriers may be prepared by well known techniques, such as those described in REMINGTON'S PHARMACEUTICAL SCIENCES, Mack Publishing Co., Philadelphia. For example, the topical carriers may be in the form of creams, oils, ointments, gels, pastes, liquids, powders, sprays, transdermal patches, and other vehicle forms suitable for application to the skin. The particular vehicles, of course, should be selected so that they do not substantially interfere with the hair growth promotion activity of the polypeptide or gene, and so that they do not cause undue side effects when administered to the patient.

The dosage or concentration of the polypeptide or gene delivery vehicle in the topical composition may vary widely. For example, the concentration of polypeptide in the topical carrier will typically be in the range from about 0.01 weight percent to 10 weight percent, typically being about 0.1 weight percent to 1 weight percent. The liposomal and/or retroviral vehicles will be present in the topical carrier usually from about 0.1 weight percent to 1 weight percent. Topical application of the compositions of the present invention to the patient's skin will result in stimulation of the emergence of hair follicles in the skin and/or stimulation of the emergence of hair from the hair follicles. It is expected that the LEF-1 gene will be incorporated into either or both of the epithelium and subadjacent mesenchyme, where gene expression will result in stimulation of hair follicle formation. Similarly, direct application of the LEF-1 polypeptide is expected to enhance hair follicle production and/or hair growth.

The following experimental data demonstrate the role of LEF-1 in hair growth.

Experimental

The following data analyze the developmental pattern of LEF-1 gene expression by *in situ* hybridization and immunohistochemistry. The role of LEF-1 in murine development was analyzed by targeted gene inactivation. Mice homozygous for the mutation in the LEF-1 gene were found to die shortly after birth and to show deficiencies in some but not all organs that express LEF-1 during their formation. Specifically, the mutant mice lack teeth, mammary glands, whiskers, and body hair, and show an absence of the mesencephalic nucleus of the trigeminal nerve (TMN). Together these findings suggest a crucial role for LEF-1 in the formation of hair organs that require inductive interactions between epithelial cells of ectodermal origin and mesenchymal cells.

Results

Expression of LEF-12 in the developing mouse

The expression of LEF-1 between E12.5 and E16.5 by *in situ* hybridization of embryo sections with an $^{35}S$-labeled LEF-1 anti-sense RNA probe. In a sagittal section of an E12.5 embryo, abundant expression of LEF-1 was detected in the mesencephalon, in the mesenchyme of the snout, and at multiple sites of organo-genesis including the ear and dental placodes (Fig. 1A). In a mid-sagittal section of an E14.5 embryo, major sites of LEF-1 gene expression were identified in the mesencephalon, tooth germs, whisker follicles, pituitary gland, and kidney (Fig. 1B). The abundance of LEF-1 gene expression is reduced markedly at E16.5, but persists at high levels in the whisker follicles (Fig. 1F) and thymus (data not shown).

Previous *in situ* hybridization studies at E10.5 identified abundant LEF-1 transcripts in the presumptive dental epithelium (Oosterwegel et al. 1993). We also observed prominent LEF-1 mRNA expression in the dental placodes of day 12.5 embryos, but found that at this stage it was confined predominantly to the condensed neural crest-derived mesenchyme underlying the ectodermally derived dental epithelium (Fig. 1C). Moreover, in the cap stage of tooth development at E14.5, expression of LEF-1 could be detected in both dental epithelium and dental papilla mesenchyme (Fig. 1D). The pattern of LEF-1 expression in the developing tooth was also confirmed at the cellular level by immunohistochemistry with purified antibodies directed against LEF-1 protein (Fig. 1E). This spatial and temporal expression pattern of LEF-1 coincides with inductive interactions between epithelial and mesenchymal cells during tooth development (Slavkin 1974; Thesleff and Hurmerinta 1981; Kollar 1983). Specifically, heterologous and heterochronic tissue recombination studies have shown that the presumptive dental epithelium has potential to induce ectopic partner tissue to form a tooth until day 12 of embryogenesis, after which time this developmental potential shifts to the condensing mesenchyme (Kollar and Baird 1970; Mina and Kollar 1987).

Higher magnification of an E12.5 embryo revealed hybridization in a defined region of the midbrain containing cells that constitute the TMN (Fig. 1G). These neurons have distinctive size and morphology and have been shown, by lineage tracing experiments in birds, to derive from the cranial neural crest (Narayanan and Narayanan 1978). Immunohistochemistry of E12.5 embryo sections with antibodies directed against LEF-1 demonstrated that the protein was expressed in cells within the TMN and immunostaining was localized predominantly to the nuclei of these cells (Fig. 1H).

Targeted inactivation of the LEF-1 gene

Using an 8-kb genomic LEF-1 DNA fragment, an insertion-type targeting gene construct in which the phosphoglycerokinase (PGK)-neo gene was inserted into the second exon of the HMG domain of LEF-1 (Fig. 2A) was generated. Previous analysis of amino- and carboxy-terminal LEF-1 polypeptides indicated that the HMG domain of LEF-1 essential for DNA binding, Giese et al. (1991) GENES & DEV. 5:2567-2578. To enrich for homologous recombinants, included a PGK-thymidine kinase (tk) gene in the plasmid, Thomas and Capecchi (1987) CELL 51:503-512. The LEF-neo-tk targeting construct was linearized and electroporated into D3 embryonic stem (ES) cells, and clones were selected doubly for the presence of the neo gene and the loss of the tk gene, Ramirez-Solis et al. (1993) ED. P.M. WASSARMAN AND M.L. DEPAMPHILIS, 225:855-877. Clones were analyzed by digesting genomic DNA with *Bam*HI and hybridizing DNA blots with a radiolabeled probe derived from genomic sequences 5' of the LEF-1 gene in the targeting vector. A 4.9-kb fragment, diagnostic of homologous recombination in the LEF-1 locus, was detected at a frequency of ~1 in 50 clones (data not shown). Accuracy of the recombination events was also confirmed by DNA blot analysis of *Bam*HI and *N*deI-digested DNA that confirmed the integrity of the 3' homologous arms and presence of a single *neo* integrant (data not shown).

Targeted ES clones were injected into C57BL/6 blastocysts, and resulting chimeric mice with ES cell contribution exceeding 70% were crossed with C57BL/6 wild-type mice. Multiple chimeras from one clone transmitted the targeted allele through the germ line Crossing the LEF-1 heterozygous mutant mice, which showed no obvious defects, generated 74 litters with 23% of the offspring homozygous for the mutation. The genotypes of the offspring were determined by DNA blot analysis of genomic DNA digested with *Bam*Hi and *Eco*RI and hybridized with a flanking probe (Fig. 2B). Homozygous mutant (-/-)

mice had a drastically impaired viability with only 63% surviving the first week after birth and only 5% surviving the second week. No homozygous mutant survived to weaning, indicating that they carry a recessive mutation in an essential gene.

Expression of LEF-1 in mutant mice

LEF-1 expression in pre-B cell lines has been shown previously, Travis et al. (1991) GENES & DEV. 5:880-894. To confirm that the targeted LEF-1 allele does not produce functional LEF-1 protein, pre-B cell lines were derived from the bone marrow was derived of 10-day old wild-type (+/+), heterozygous (+/-), and homozygous mutant (-/-) mice by transformation with Abelson murine leukemia virus (MuLV). Nuclear extracts from the pre-B cell lines were prepared and assayed for the presence of LEF-1 protein by immunoblot analysis (Fig. 2C). Incubation of the immobilized protein extracts with antibodies directed against LEF-1 protein allowed for detection of 55- and 39-kD LEF-1 polypeptides in pre-B cells from +/+ and +/- mice. In contrast, these polypeptides were not detected in pre-B cell extracts from -/- mice, indicating that the mutation in the LEF-1 gene eliminates its proteins expression.

Arrested follicle development in body hair and whiskers

The most obvious phenotype of the LEF-1 deficient mice was the lack of body hair and vibrissae (whiskers) (Fig. 3). In homozygous mutant mice, some rudimentary hair without pigmentation became visible at approximately day 9 after birth but was progressively lost after day 12. Moreover, the mutant mice had a pointed snout and were significantly smaller than sibling heterozygous and wild-type mice.

Hair follicle development in the mouse normally starts between E13 and E14 with the formation of small focal epidermal thickenings, termed placodes, in

association with small dense aggregates of mesenchyme, termed dermal papilla, Sengel (1976) TRENDS GENET. 8:55-61; Hardy (1992) THE SECRET LIFE OF THE HAIR FOLLICLE. Subsequently, the epidermal placodes grow into the underlying dermis and by E18, most nascent follicles have acquired characteristics of a mature follicle.

Histological examination of the skin of LEF-1 deficient embryos at E14 and E16 revealed that follicle formation began at the expected time and proceeded through the early stages, but the number of follicles was reduced to one-third of that found in wild-type mice (data not shown). In 3-day-old homozygous mutant mice, however, virtually all follicles present were short and rudimentary, suggesting an arrest in development at a stage corresponding to E17 (Fig. 4A-D). Moreover, the skin of the mutant mice was deficient in dermal fat and the follicles lacked melanin. Because LEF-1 is expressed in neural crest-derived cells, the skin was examined for the presence of melanocytes, which are of neural crest origin. Immunohistochemistry was performed on sections of skin with an antibody directed against the melanocyte-specific antigen c-kit, Nishikawa et al. (1991) EMBO J. 10:2111-2118. Melanocytes were detected in both wild-type and mutant skin (Fig. 4E,F), suggesting that the absence of melanin in the skin of LEF-1 deficient mice may represent a functional defect secondary to the arrest in hair follicle development, Billingham and Silvers (1969) Q. REV. BIOL. 35:1-41.

The defect in hair development was most pronounced in the whiskers, which normally are much larger and develop 2-3 days earlier than other hair on the body. No whiskers were detected in postnatal mutant animals at day 3. The lack of whiskers and their associated dermal fat seemed to account for the pointed appearance of the animals' snouts, because the mandible and maxillae were structurally normal.

The histological examination was extended to other epidermal appendages such as nails and sweat glands. Nails and histologically normal sweat glands were detected in the LEF-1 -/- mice at 10 days after birth (data not shown) indicating that only some skin structures are affected by mutation of the LEF-1 gene.

Materials and methods

Construction of the LEF-1 targeting vector and cell culture

A mouse genomic library from 129/Sv mice was screened with an LEF-1 cDNA probe encompassing sequences that encode the DNA-binding domain of LEF-1. One phage was isolated that included the second exon of the HMG domain (nucleotides 1899-2119 of the LEF-1 cDNA). An 8-kb genomic LEF-1 fragment was excised with *Sal*I and *Xho*I and subcloned into a Bluescript vector (Stratagene) that lacked the *Sma*I (*Xma*I) site. Insertion of a filled-in *Eco*RI-*Bam*HI fragment containing the PGK-neo gene into the filled-in *Xma*I site of the 8-kb LEF-1 subclone generated an insertion type LEF-1 targeting vector. Finally, an *Eco*RI-*Hin*dIII fragment containing the PGK-tk gene was inserted into the targeting vector to generate the final gene construct used for homogenous recombination in ES cells.

For electroporation of embryonic stem cells, the targeting vector was linearized with *Cla*I. Electroporation of ES cells and selection of G418- and FIAU- (from Bristol-Myers Squibb) resistant colonies were performed as described by Ramirez-Solis et al. (1993) Ed. P.M. Wassarman and M.L. DePamphilis, 225:855-877, with the exception that G418-resistant primary embryonic fibroblasts were used as feeders. For DNA blot analysis, genomic DNA was prepared as described by Ramirez-Solis et al. (1993), *supra*.

DNA blot analysis

Genomic DNA was isolated from the livers of E16 embryos and 20μg of each sample was digested with *Eco*RI and *Bam*HI. The fragments were separated on a 0.9% agarose gel and transferred to GeneScreen Plus (DuPont). The probe, a 180-bp *Sty*I-*Xho*I fragment from the LEF-1 locus, was labeled with ($\alpha$-$^{32}$P)dCTP by random priming (Boehringer Mannheim) and hybridized overnight at 42°C. Blots were washed to a final stringency of 0.1 x SSC, 0.1% SDS at 65°C, and exposed to film overnight.

Immunoblot analysis

Pre-B cell lines were generated from bone marrow by transformation with Abelson MuLV. Nuclear extract from $5x10^7$ cells of each pre-B cell line were separated on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose. The blot was incubated with a rabbit anti-murine LEF-1 antibody, Travis et al. (1991) GENES & DEV. 5:880-894 and developed with an alkaline phosphatase-conjugated secondary antibody (Boehringer Mannheim).

In situ hybridizations

Embryos were fixed in 4% (wt/vol) paraformaldehyde in phosphate-buffered saline (PBS) for 24 hours at 4°C, immersed in 20% (wt/vol) sucrose/PBS for 24 hours at 4°C, frozen in powdered dry ice, and then stored at -80°C until cryostat sectioning. Serial sections of 14- to 16μm thickness were thaw-mounted onto microscope slides (Superfrost Plus, Fisher) and stored at -80°C until use.

RNA expression vectors containing 0.36-kb protein-coding region of the LEF-1 cDNA (nucleotides 1158-1517; Travis et al. 1991) were used to generate $^{35}$S-labeled sense and antisense RNA probes as described by Tecott et al. (1993) PROC. NATL. ACAD. SCI. 90:1430-1434. Embryo sections were hybridized, washed, and developed essentially as described by Tecott et al. (1993).

Hybridizations were carried out at 52°C in 50% (vol/vol) formamide, 0.3 M NaCl, 20mM Tris-HCl (pH 8.0), 5 mM EDTA, 1 X Denhardt's solution, 10 mM $NaH_2PO_4$, 10 mM dithiothreitol, 0.5 mg/ml of yeast tRNA, 10% (wt/vol) dextran sulfate. After hybridization, sections were rinsed in 2 X SSC, 10 mM 2-mercaptoethanol, 1 mM EDTA at room temperature and then incubated in RNase (30 mg/ml), 0.5 M NaCl, 10 mM Tris-HCl (pH 8.0), for 40 min. at 37°C. A high-stringency wash was then performed in 0.1 X SSC, 10 mM 2-mercaptoethanol, 1 mM EDTA for 2 hours at 55°C. The slides were rinsed in 0.5 X SSC, dehydrated through a graded ethanol series containing 0.3 M ammonium acetate, air dried, and dipped in Kodak NTB2 emulsion, diluted 1:1 with $H_2O$. The emulsion was developed in Kodak D19 developer, and sections were counterstained with cresyl violet.

Histological procedures

All postnatal animals were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2-7.4). Embryos were dissected out and fixed by immersion in the same fixative solution. Fixed newborn mice and embryos were dehydrated in ethanol, embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin-eosin for conventional analysis. Brains from older animals (P9-P10) were also embedded and paraffin sections were Nissl stained using 0.1% cresyl violet. Some tissues were postfixed with 2% osmium tetroxide in phosphate buffer, dehydrated, and embedded in araldite resin (Durcupan, Fluka). One-micron-thick sections were obtained in a Reichert Ultracut E and stained with 1% toluidine blue.

For immunocytochemistry using antibodies against $p75^{LNGFR}$, brains of P9-P10 mice were dissected out from perfused animals and left in the same fixative overnight. Sagittal sections at 60 μm were obtained with a vibratome and collected in TBS [10 mM Tris (pH 7.5),

150 mM NaCl]. Sections were incubated in 3% hydrogen peroxide and 10% methanol, to quench endogenous peroxidase activity, blocked 2 hours in a TBS solution containing 0.4% Triton X-100, 1% glycine, 3% bovine serum albumin, and 10% normal goat serum, and left overnight at 4°C in the same blocking solution containing polyclonal antibodies against $p75^{LNGFR}$ at 1μg/ml. The ABC method (ABC Elite kit, Vector Labs) was used for immunodetection. Briefly, after washing the sections several times with blocking solution, they were incubated for 1 hour in biotinylated secondary anti-rabbit antibodies at 1:200, washed several times, and incubated for an additional hour in avidin-biotin-peroxidase complex, 1:300. Peroxidase was then reacted with 0.05% diaminobenzidine tetrahydrochloride and 0.003% hydrogen peroxide in 0.1 M Tris buffer (pH 7.5).

Melanocytes were stained with a rat monoclonal antibody against c-kt (ACKZ; Nishikawa et al. (1991) EMBO J. 10:2111-2118. Pieces of skin from the back of newborn mice were dissected, fresh frozen in OCT medium (Tissue Tek) using dry ice, sectioned at 10 μm in a cryostat, and thaw mounted on poly-L-lysine coated slides. Sections were fixed with cold acetone for 1 minute and probed with c-kit antibody as described above.

Although the foregoing method has been described in some detail by way of illustration and example, for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Regents of the University of California

(ii) TITLE OF INVENTION: Methods and Compositions for Hair Growth Promotion

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Robbins, Berliner & Carson
(B) STREET: 201 N. Figueroa Street, 5th Floor
(C) CITY: Los Angeles
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 90012-2628

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Berliner, Robert
(B) REGISTRATION NUMBER: 20,121
(C) REFERENCE/DOCKET NUMBER: 5555-419

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 213-977-1001
(B) TELEFAX: 213-977-1003

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2460 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGGAGGAGCC GAGGGGCCAC CGGCAGCTCT TTGCTTTGAC CGAGGGGAGG CGGGGAGAGA 60

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GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTAAGTGTGT GTATCGGCCC GAGCTGCGGG 180

CTGGGACATT TGGGCCCGAA GCTCCTGCTG TGACTCCCCA AGACTCCGCC GTGCCAGCCA 240

CCGCCGATTC CCAGCGCTCA TCATCACAAA CTTTATTCTT GGCAAACTTC TCTTTTTCTC 300

CCCTCCCCCT CCAGCAGATT AAATGCTCCT CCAGAAGGAA AACCGAAGCG AAAGGGAAGG 360

AAAGAAGCTC TAACGCGGAC GTCTGCAGCC CGGTGGCTCT TTATTGTTTA CTCTGAAGGA 420

AGTGGACTTT TCGGTATTTT CTGATTCTTC TCGTACCTCC GCTGGGGCAA AGGGAGCCTC 480

TTGGCCAGCT CTCCTCTTCT CAAAAACAAA ACAAAACAAA ACCCCAAATC ACCGAAATCA 540

GCATCCGAGG AATCCGTGGA GAGGTTTTGC ACGGTGACGC ACCACGGGAC CCTCGTTTGC 600

ACTTTGGCAA GTCGTGTCCC CTCCGGAACC GGTGTCCTCG AAGCACAGAC CCCAGCAACG 660

AGTGCGGCGT GGGGTGGCCA CCCAGCCCTG GCAGCCTAGC CTAGTGCACG CGGAGCGCGT 720

AGACGCTCTC AGAGGCGCCG GCGTGCCGCC CTCGCAGTCT GGTGCCCTTC GCCCCAGGCC 780

GGGGCGCCGG CGTCCTTCTA AGTGGGAAAG CGCGGCAATC GCAGAGGCTC CTGCAGCTGC 840

TCCAGCCGTG GGCCCCTCGC TTGCCCACCC GGCTTCCATT CACAGTCCTC CCGCACTCAG 900

TCTTCCAACT CCCCCACCTC ACATCGACCC TCCCTTCTTG CTGTCTCGGA GTGCTCCCGC 960

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TCAAGTCATC TTTGGTTAAC GAGTCCGAAA TCATCCCAGC CAGCAACGGG CATGAGGTGG 1200

TCAGACAAGC CCCGTCCTCT CAGGAGCCCT ACCACGACAA GGCCAGAGAA CACCCTGATG 1260

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CACCCATCCC GAGGACATCA AATAAAGTGC CCGTGGTGCA GCCCTCTCAC GCGGTCCACC 1440

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ACATCCCGTC AGATGTCAAC TCCAAGCAAG GCATGTCCAG ACACCCTCCA GCTCCTGAAA 1560

TCCCCACCTT CTACCCCCTG TCTCCGGGCG GCGTTGGACA GATCACCCCA CCCATTGGCT 1620

GGCAAGGTCA GCCTGTTTAT CCCATCACGG GTGGATTCAG GCAACCCTAC CCATCCTCAC 1680

TGTCAGGCGA CACTTCCATG TCCAGGTTTT CCCATCATAT GATTCCTGGT CCCCCTGGCC 1740

CCCACACAAC TGGCATCCCT CATCCAGCTA TTGTAACACC TCAGGTCAAA CAGGAGCACC 1800

CCCACACGGA CAGTGACCTA ATGCACGTGA AGCCTCAACA CGAACAGAGA AAGGAGCAGG 1860

AGCCCAAAAG ACCTCATATT AAGAAGCCTC TGAATGCTTT CATGTTATAT ATGAAAGAAA 1920

TGAGAGCGAA TGTCGTAGCT GAGTGCACGC TAAAGGAGAG TGCAGCTATC AACCAGATCC 1980

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GCAAGAAGAA GAAGAGGAAG AGAGAGAAGC TACAGGAGTC GACTTCAGGT ACAGGTCCCA 2160

GAATGACAGC TGCCTACATC TGAAACATGG TGGTAAGAGA AGCTCCTTCC CAACGTGCAA 2220

AGCCAAGGCA GCGACCCCAG GCCCTCTTCT GGAGATGGAA GCTTGTTGAA ACCCCAGACT 2280

GTCTCCACAG CTTGCCCGGC TGACCCCAAG GAACACTGAC AGCAACCTTA CCCTGAGGTC 2340

ACTGCTAGCG CTGACCCGAA GACACAGTCA CTGCCACCTC TTCCTTCTGT CGTCTACTGC 2400

AAGCGCCGAC TTCCAAAAAG AAAGCCGAAA ACGGTTGTTT CGGAAAAAAA AAAAAAAAAA 2460

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 397 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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            20                  25                  30

Glu Lys Ile Phe Ala Glu Ile Ser His Pro Glu Glu Glu Gly Asp Leu
        35                  40                  45

Ala Asp Ile Lys Ser Ser Leu Val Asn Glu Ser Glu Ile Ile Pro Ala
    50                  55                  60

Ser Asn Gly His Glu Val Val Arg Gln Ala Pro Ser Ser Gln Glu Pro
65                  70                  75                  80

Tyr His Asp Lys Ala Arg Glu His Pro Asp Glu Gly Lys His Pro Asp
                85                  90                  95

Gly Gly Leu Tyr Asn Lys Gly Pro Ser Tyr Ser Ser Tyr Ser Gly Tyr
            100                 105                 110

Ile Met Met Pro Asn Met Asn Ser Asp Pro Tyr Met Ser Asn Gly Ser
        115                 120                 125

Leu Ser Pro Pro Ile Pro Arg Thr Ser Asn Lys Val Pro Val Val Gln
    130                 135                 140

Pro Ser His Ala Val His Pro Leu Thr Pro Leu Ile Thr Tyr Ser Asp
145                 150                 155                 160

Glu His Phe Ser Pro Gly Ser His Pro Ser His Ile Pro Ser Asp Val
                165                 170                 175

Asn Ser Lys Gln Gly Met Ser Arg His Pro Pro Ala Pro Glu Ile Pro
            180                 185                 190

Thr Phe Tyr Pro Leu Ser Pro Gly Gly Val Gly Gln Ile Thr Pro Pro
        195                 200                 205

Ile Gly Trp Gln Gly Gln Pro Val Tyr Pro Ile Thr Gly Gly Phe Arg
    210                 215                 220

Gln Pro Tyr Pro Ser Ser Leu Ser Gly Asp Thr Ser Met Ser Arg Phe
225                 230                 235                 240

Ser His His Met Ile Pro Gly Pro Pro Gly Pro His Thr Thr Gly Ile
                245                 250                 255

Pro His Pro Ala Ile Val Thr Pro Gln Val Lys Gln Glu His Pro His
            260                 265                 270

Thr Asp Ser Asp Leu Met His Val Lys Pro Gln His Glu Gln Arg Lys
        275                 280                 285

Glu Gln Glu Pro Lys Arg Pro His Ile Lys Lys Pro Leu Asn Ala Phe
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Met Leu Tyr Met Lys Glu Met Arg Ala Asn Val Val Ala Glu Cys Thr
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                325                 330                 335

Ala Leu Ser Arg Glu Glu Gln Ala Lys Tyr Tyr Glu Leu Ala Arg Lys
            340                 345                 350

Glu Arg Gln Leu His Met Gln Leu Tyr Pro Gly Trp Ser Ala Arg Asp
        355                 360                 365

Asn Tyr Gly Lys Lys Lys Lys Arg Lys Arg Glu Lys Leu Gln Glu Ser
    370                 375                 380

Thr Ser Gly Thr Gly Pro Arg Met Thr Ala Ala Tyr Ile
385                 390                 395
```

**WHAT IS CLAIMED IS:**

1. A method for promoting hair growth, said method comprising restoring or enhancing the activity of LEF-1 in hair follicles.

2. A method for promoting hair growth as in claim 1, said method comprising:

providing a composition including LEF-1 or biologically equivalent substance, a topical carrier in an amount sufficient to restore or enhance hair growth in hair follicles; and

applying said composition to a region of skin where hair growth promotion is desired.

3. A method as in claim 2, wherein the LEF-1 is full length LEF-1 protein.

4. A method as in claim 3, wherein the LEF-1 is recombinant LEF-1 protein.

5. A method for promoting hair growth as in claim 1, said method comprising:

providing a composition comprising an LEF-1 gene in a topical carrier; and

applying said composition to a region of skin where hair growth promotion is desired.

6. A method as in claim 5, wherein the LEF-1 gene is present in a liposomal or retroviral vector.

7. A method as in claim 6, wherein the LEF-1 gene is in reading from with homologous or heterologous regulatory control sequences.

8. A composition for promoting hair growth, said composition comprising an LEF-1 polypeptide or LEF-1 gene in a topical carrier.

9. A composition as in claim 8, wherein the LEF-1 polypeptide is full length LEF-1 protein.

10. A composition as in claim 9, wherein the full length LEF-1 protein is recombinant LEF-1 protein.

11. A composition as in claim 8, wherein the LEF-1 gene is present in a liposomal or retroviral vector.

12. A composition as in claim 11, wherein the composition further comprises homologous or heterologous regulatory control sequences in reading frame with the LEF-1 gene.

FIG. 1-A

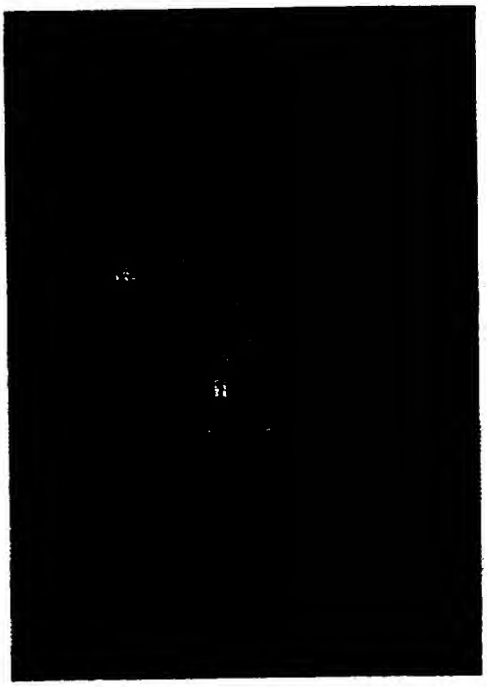

FIG. 1-B

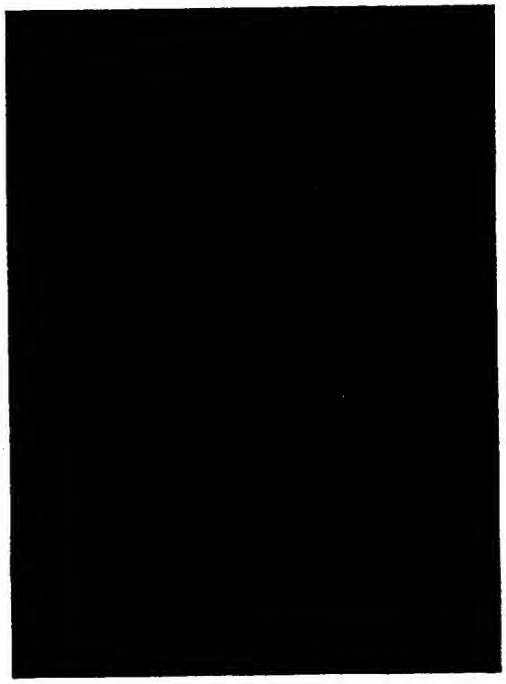

FIG. 1-C

FIG. 1-D

FIG. 1-E

FIG. 1-F

FIG. 1-G

FIG. 1-H

FIG. 2-A

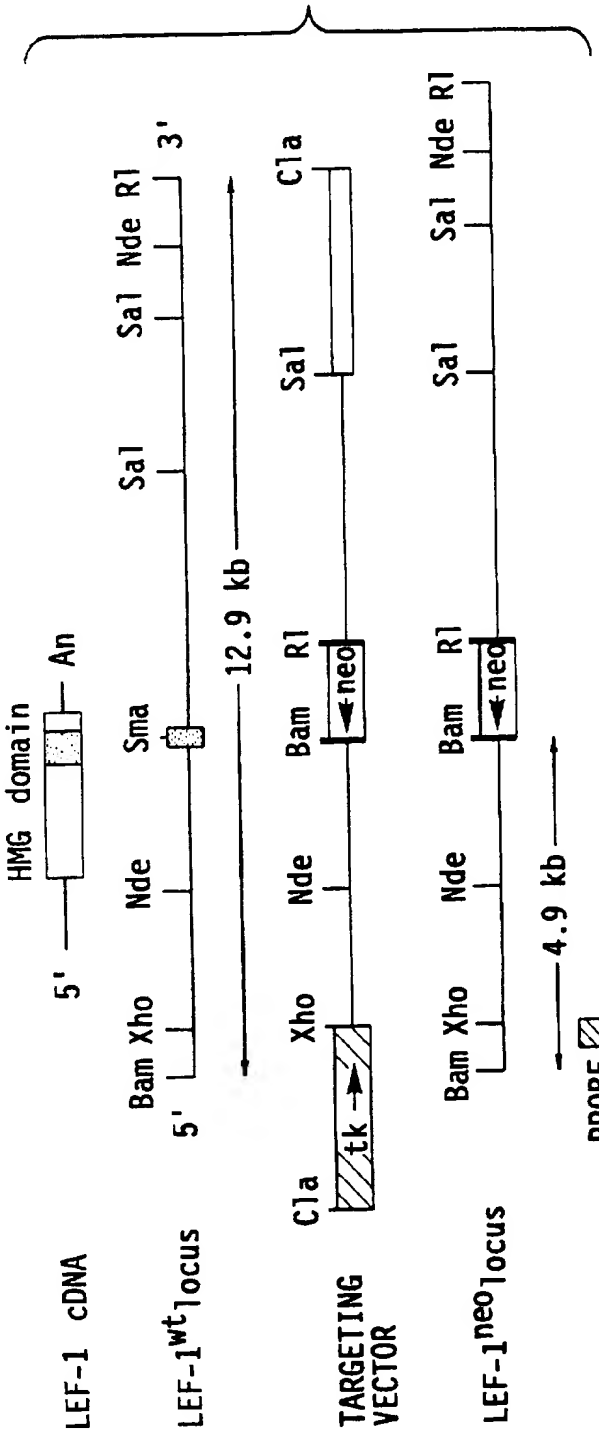


FIG. 2-B

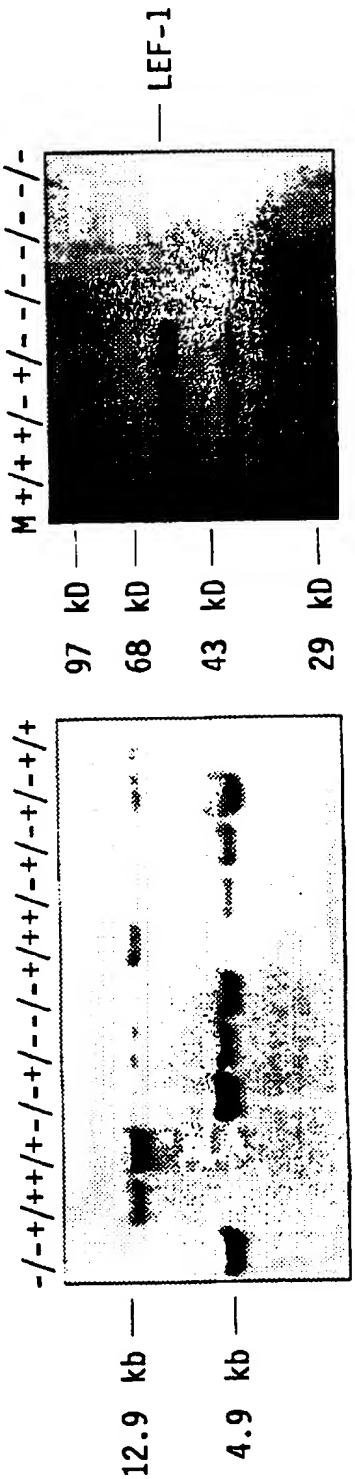


FIG. 2-C

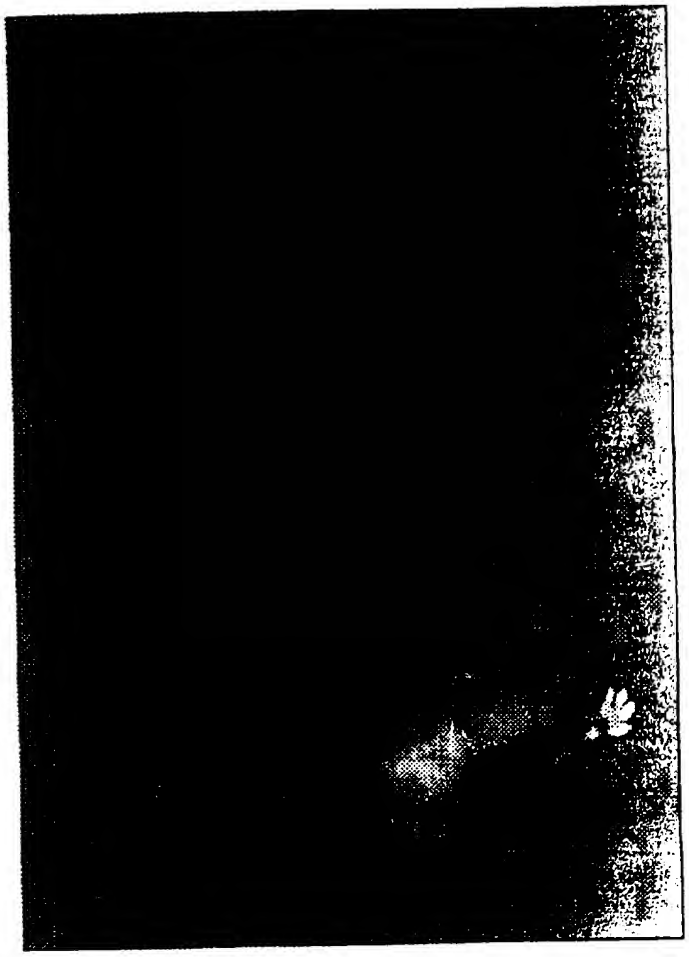

FIG. 3

FIG. 4-A

FIG. 4-B

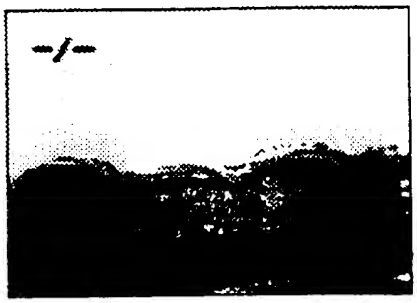

FIG. 4-C


FIG. 4-D


FIG. 4-E

FIG. 4-F

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17928

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 38/00, 38/17; C07K 14/47; C12N 15/12
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2; 536/23.1, 23.5; 530/300, 350; 435/69.1, 69.7, 252.3, 240.1, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAPLUS, BIOSIS, EMBASE, MEDLINE, CONFSCI
search terms: LEF-1, lymphoid enhancer factor 1, hair development, transcription factor, hair growth

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ZHOU et al. Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. Genes & Devel. 1995, Vol. 9, pages 700-713, entire document. | 1-4, 8-10 |
| X<br>---<br>A | GIESE et al. LEF-1 contains an activation domain that stimulates transcription only in a specific context of factor-binding sites. EMBO J. 1993, Vol. 12, No. 12, pages 4667-4676, especially pages 4667-4675. | 1-4, 8-10<br>---<br>5-7, 11-12 |
| X<br>---<br>A | VAN GENDEREN et al. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. Genes & Devel. 1994, Vol. 8, pages 2691-2703, entire document. | 1-4, 8-10<br>---<br>5-7, 11-12 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>10 DECEMBER 1996 | Date of mailing of the international search report<br>14 FEB 1997 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>KENNETH A. SORENSEN<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17928

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-10; Claims 11 and 12 could be searched without effort justifying an additional fee.

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17928

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/2, 44; 536/23.1, 23.5; 530/300, 350; 435/69.1, 69.7, 252.3, 240.1, 320.1

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-4, 8-10, drawn to a method for promoting hair growth comprising providing LEF-1, and a composition comprising LEF-1 in a topical carrier.
Group II, claim(s) 1, 5-7, drawn to a method for promoting hair growth comprising providing a LEF-1 gene, and a composition comprising a LEF-1 gene in a topical carrier.
Group III, claim(s) 8,11,12, drawn to a LEF-1 nucleic acid composition.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The claims of Group I are drawn to an invention whose special technical feature is the LEF-1 protein and Group I consists of claims drawn to a method of promoting hair growth comprising providing the LEF-1 protein, and a composition comprising LEF-1 in a topical carrier, whereas the special technical feature of the invention of Group II is the LEF-1 gene and consists of claims for a method of gene therapy comprising providing a LEF-1 gene, and compositions comprising the LEF-1 gene in a topical carrier. The protein of Group I and the gene of Group II each have materially different chemical structures and materially different functional properties. The invention of Group III, under CFR 1.475(d) which states that when multiple products, processes of manufacture, or uses are claimed, the first invention of the category first mentioned in the claims of the application and the first recited invention of each of the other categories related thereto will be considered as the main invention in the claims, is considered not to share a special technical feature with Groups I and II. These chemical structures and functional properties are the special technical features that identify each invention and distinguish each invention from the others, because none of the special technical features is shared by the separate groups. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

Because a search of group III does not justify an additional fee, it has been searched along with Group II.